Support to the Global TB Drug Facility to develop a mechanism for supplying Laboratory Kits for TB diagnosis to high burden countries

Trip Report

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Catherine Mundy

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Rational Pharmaceutical Management Plus Program
Center for Pharmaceutical Management
Management Sciences for Health
4301 North Fairfax Drive, Suite 400
Arlington, VA 22203 USA
Phone: 703-524-6575
Fax: 703-524-7898
E-mail: rpmplus@msh.org

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About RPM Plus

RPM Plus works in more than 20 developing countries to provide technical assistance to strengthen drug and health commodity management systems. The program offers technical guidance and assists in strategy development and program implementation both in improving the availability of health commodities—pharmaceuticals, vaccines, supplies, and basic medical equipment—of assured quality for maternal and child health, HIV/AIDS, infectious diseases, and family planning and in promoting the appropriate use of health commodities in the public and private sectors.

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Key Words and Terms

Tuberculosis, Laboratory diagnosis, Sputum smear microscopy, Ziehl-Neelsen stain

Contents

Abstract

The Global TB Drug Facility (GDF) as part of its mandate to accelerate DOTS expansion decided to formulate and develop TB diagnostic laboratory kits to be made available to countries with high disease burden. Management Sciences for Health (MSH) was requested to provide the services of a consultant to **develop a mechanism to enable the GDF to supply high disease burden countries with laboratory equipment and consumables required for diagnosis of tuberculosis.**

Activities:

Lists of contents plus individual item specifications and quantities were prepared for the following laboratory commodities:

i. Consumables Kit A (with ready-to-use Ziehl-Neelsen stains)
ii. Consumables Kit B (with chemicals and pre-weighed dry reagents for in-country preparation of Ziehl-Neelsen stains)
iii. Sputum collection containers
iv. Equipment Starter Kit A (to be used in conjunction with Consumables Kit A)
v. Equipment Starter Kit B (to be used in conjunction with Consumables Kit B)
vi. Microscope kit

The current GDF application form for drugs was modified and adapted to produce an application form for laboratory kits. Algorithms were devised to enable applicants to quantify order requirements for Consumables Kits, based on number of smear positive cases detected and smear positivity rate. Specifications on the composition of individual stains, methods for preparing stains and standard laboratory procedures for sputum smear microscopy were prepared in line with WHO guidelines.

In order to complete the development of the proposed laboratory kits, immediate follow-up activities to be arranged by the GDF, were discussed and agreed with the GDF Technical Officer for Procurement. These included the need to obtain financial support for the laboratory kits initiative and field testing and evaluation of the kits prior to finalising the kit specifications and the tendering and procurement process.

Summary of Recommendations made to the GDF for consideration:

1. The priority recipients for laboratory kits should be resource-poor countries with high TB burden, which have particular difficulties in obtaining laboratory equipment and consumables.
2. Applicants for laboratory kits to be asked to provide information on their AFB smear microscopy guidelines, quality assurance and laboratory supervisory mechanisms and proposed in-country management of laboratory kits. Where these systems are lacking, applicants to be requested to ensure that they are in place within an agreed time scale. Particular difficulties to be referred to the Stop-TB secretariat to request technical assistance from partners and donors.

3. Only the Consumables Kit providing ready-to-use stains and its associated Equipment Starter Kit to be supplied in the first instance. Other kit types only to be made available after further assessments for demand and feasibility of production.
4. The microscope kit to be supplied on the understanding that the applicant is responsible to make the necessary local arrangements for cleaning, maintenance and professional servicing.
5. The GDF to co-opt a team of independent assessors to review all applications for laboratory kits and to visit individual countries following the review of their applications.
6. The GDF to institute a system, similar to that used for drugs, to assess the ongoing performance of each applicant for laboratory kits. This will include the requirement for regular written reports as well as country assessment visits to monitor the operation and management of TB laboratory services.
7. In order to obtain the necessary technical assistance and financial resources required to launch and sustain this new initiative, the GDF should request partners and donors already supporting its activities to extend their support to encompass that required for the laboratory kit initiative. This additional support will be particularly important for the assessments and country visits.

Acronyms

AFB	Acid fast bacilli
CDC	Centers for Disease Control and Prevention, Atlanta
cm	Centimetre
DC	Direct current
DOTS	WHO TB Control strategy
GDF	Global TB Drug Facility
GFATM	Global Fund for AIDS, TB and Malaria
GNP	Gross National Product
HCl	Hydrochloric acid
IUATLD	International Union Against Tuberculosis and Lung Disease
l	Litre
m	Metre
mA	Milliamps
ml	Millilitres
mm	Millimetres
MSH	Management Sciences for Health Inc.
NA	Numerical aperture
NGO	Non-Governmental Organisation
PHC	Primary health care
QC	Quality control
TB	Tuberculosis
US$	United States Dollar
USAID	United States Agency for International Development
V	Volts
WHO	World Health Organization
ZN	Ziehl Neelsen

Background

The Global TB Drug Facility (GDF) was established by the Stop TB Global Partnership on 24 March 2001 as a new initiative for increasing access to, and availability of high quality TB drugs and to accelerate DOTS expansion. The GDF is housed in WHO headquarters in Geneva and managed by a small team in the Stop TB partnership secretariat.

Whilst it is well-recognised that drugs are essential to TB prevention and cure, their proper use depends on the availability of reliable, quality-assured laboratory diagnosis of TB. Sputum smear microscopy for TB diagnosis, an integral component of the DOTS strategy, is a relatively simple laboratory procedure but is often hampered by the lack of appropriate diagnostic equipment and sustainable supplies of quality laboratory consumables.

In 2003, the GDF, as part of its mandate to accelerate DOTS expansion, carried out some preliminary research to investigate the possibility of supplying laboratory equipment and reagents to countries with high TB burden. As a result of these investigations, the GDF decided to proceed with the formulation and development of diagnostic laboratory kits with the intention of making supplies of these available to countries with high disease burden.

In February 2004, the Executive Secretary of the Stop TB Partnership Secretariat requested Management Sciences for Health (MSH), which already assists the GDF with drug management, to assist the GDF with this work by providing the services of a laboratory consultant. MSH assigned Catherine Mundy, Principal Program Associate for Laboratory Services, to work with the GDF in Geneva for one week with the following objective:

Objective

To develop a mechanism to enable the GDF to supply high disease burden countries with laboratory equipment and consumables required for diagnosis of tuberculosis.

Scope of Work

To determine the contents and specifications for the proposed GDF diagnostic kits:

- Starter Kit
- Microscope Kit
- Consumables Kit containing dry reagents and pure solvents
- Consumables Kit containing ready-to-use stains

Activities

In order to achieve the objective and complete the scope of work, the following activities were undertaken:

1. Review of the preliminary research results carried out by the GDF in 2003, including survey respondents' opinions on laboratory kits.

2. Review of the GDF structure and current mechanisms for procuring and supplying TB drugs to countries.

3. Daily meetings with the GDF Technical Officer for Procurement (seconded from MSH to GDF) to discuss GDF requirements, the operational aspects of supplying laboratory kits and their in-country distribution and use.

4. Preparation of list of contents plus individual item specifications and quantities for the following laboratory commodities:
    i. Consumables Kit A (with ready-to-use Ziehl-Neelsen stains)
    ii. Consumables Kit B (with chemicals and pre-weighed dry reagents for in-country preparation of Ziehl-Neelsen stains)
    iii. Sputum collection containers
    iv. Equipment Starter Kit A (to be used in conjunction with Consumables Kit A)
    v. Equipment Starter Kit B (to be used in conjunction with Consumables Kit B)
    vi. Microscope kit

   See **ANNEX 1** for details

5. Modification and adaptation of the current GDF application form for drugs to produce an application form for laboratory kits.
   See **ANNEX 2** for details

6. Preparation of algorithms to enable countries to quantify order requirements for Consumables Kits based on number of smear positive cases detected and smear positivity rate.
   See **ANNEX 3** for details

7. Specifications on the composition of individual stains and methods for preparing these, in line with WHO guidelines. This information to be provided by GDF to prospective manufacturers/suppliers of Consumables Kits
   See **ANNEX 4** for details

8. Preparation of standard laboratory procedures for TB microscopy using Ziehl-Neelsen staining method, in line with WHO/IUATLD recommendations, copy to be included in each Consumables Kit.
   See **ANNEX 5** for details

Beneficificiaries, Collaborators and Partners

This work was undertaken in partnership with the GDF and in direct consultation with the Technical Officer for Procurement as counterpart. The immediate beneficiary of this work is the GDF. The documents detailed in Annexes 1 – 5 are intended to provide sufficient information to enable the GDF team to proceed with the tendering and procurement of the designated kits.

In the broader context, the provision of laboratory kits is expected to facilitate DOTS expansion worldwide by ensuring the provision of standardised, quality laboratory consumables and equipment.

Next Steps

In order to complete the development of the proposed laboratory kits and to make supplies of these available worldwide, it was discussed and agreed with the Technical Officer for Procurement that the GDF would be responsible to arrange for a series of immediate follow-up activities, as outlined below:

- Engage services of computer expert to modify format of *Application Form for Laboratory Kits* for easy 'on-line' web site use.
- Seek financial support from donors for the laboratory kits initiative.
- Engage services of one or more commercial companies to make up a small number of sample kits for field evaluation.
- Arrange for field testing of Consumables Kit A, Equipment Starter Kit A and the Microscope Kit, preferably at district level in a minimum of three contrasting sites.
- On-site evaluation of field tests by laboratory specialists, followed by modification of kits contents and specifications as required.
- GDF expert review and agreement on final specifications for each kit type.
- Tendering and procurement for kits in line with GDF regulations and procedures.

Recommendations

The current internationally recommended five-point TB Control DOTS strategy specifies case detection among symptomatic patients using sputum smear microscopy as the entry point for anti-TB drug therapy. Whilst the provision of laboratory kits worldwide through the GDF is expected to facilitate the supply of all essential equipment and consumables required for sputum smear microscopy, it is important to point out that several other requirements also need to be in place to achieve quality-assured microscopy results. Adequate numbers of suitably trained and well motivated staff, safe laboratory premises with adequate working space, management of equipment and consumable supplies, quality monitoring and regular supervision are all pre-requisites for reliable and sustainable sputum smear microscopy services.

Whilst the development of the TB laboratory network in individual countries is beyond the remit and scope of the GDF, the proposed mechanism for supplying laboratory kits provides an opportunity to monitor not only the need for such kits and how they are being utilised but also whether appropriate management support systems are in place so that the use of these kits can contribute to improving the availability of quality-assured TB diagnosis.

With this in mind the following recommendations are made to the GDF for consideration:

1. The priority recipients for laboratory kits should be resource-poor countries with high TB burden, which have particular difficulties in obtaining laboratory equipment and consumables. These will include countries in conflict and those with large refugee and displaced populations.

2. In addition to the basic information and requirements for GDF support to supply TB drugs, each country seeking support for laboratory kits should be asked to provide the following as part of the Application for Laboratory Kits:

   - Copy of national guidelines for AFB smear microscopy
   - Documentation on AFB quality assurance and laboratory supervisory mechanisms
   - Documentation describing proposed flow and management of laboratory kits within the country
   - Country information and statistics on the number and type of microscopy services and laboratory workload

   Applicants should be requested to ensure that essential management systems for the laboratory network are in place within a specified and agreed time scale. Where particular difficulties are identified and/or if specific technical assistance is required, their needs should be referred to the Stop-TB secretariat in order that the necessary assistance can be sought from other partners and donors.

3. Although two types of Consumables Kits have been recommended (A and B – see Annex 1), it is suggested that the kit providing ready-to-use stains and its associated Equipment Starter Kit are supplied in the first instance. The kit containing solvents and pre-weighed dry reagents should only be made available later after determining the feasibility of production and following the initial country evaluations of kit use and country demands for such a kit.

4. In order to ensure value for money and several years use, the microscope kit should only be supplied on the understanding that the applicant is responsible to make the necessary local arrangements for cleaning, maintenance and professional servicing.

5. The GDF to co-opt a team of independent assessors to review all applications for laboratory kits and to visit individual countries following the review of their applications.

6. The GDF to institute a system, similar to that used for drugs, to assess the ongoing performance of each applicant for laboratory kits. This should include the requirement for regular written reports to be submitted to the Stop TB Secretariat as well as country assessment visits by co-opted laboratory consultants to monitor the management and operation of TB laboratory services. Where it can be arranged, it is preferable for a team of assessors to monitor drug and laboratory kit management in any particular country at the same time.

7. In order to obtain the necessary technical assistance and financial resources required to launch and sustain this new initiative, the GDF should request partners (e.g. MSH and others) and donors (e.g. USAID and others) already supporting GDF activities to extend their support to encompass that required for the laboratory kit initiative. This additional support will be particularly important for the assessments and country visits.

ANNEX 1.

CONTENTS, SPECIFICATIONS AND QUANTITIES FOR LABORATORY KITS

LABORATORY DIAGNOSTIC KITS

To promote DOTS expansion in low-income countries (GNP < US$3000) with high burden of TB, the Global TB Drug Facility will provide laboratory diagnostic kits to eligible countries free of charge. The kits will also be available for purchase at a reasonable cost to other partners who do not meet the eligibility criteria for free kits.

The following will be made available:

- Consumables Kit A (with ready-to-use Ziehl-Neelsen stains)
- Consumables Kit B (with chemicals and pre-weighed dry reagents for in-country preparation of Ziehl-Neelsen stains)
- Sputum collection containers
- Equipment Starter Kit A (to be used in conjunction with Consumables Kit A)
- Equipment Starter Kit B (to be used in conjunction with Consumables Kit B)
- Microscope kit

1. CONSUMABLES KIT A

Contains materials sufficient to prepare and stain 1000 sputum smears

	Item ?	***Quantity per kit***	***Specifications/Use***
1	Ready – to use Ziehl-Neelsen stains*:*		Minimum expiry date of 2 years from time of delivery
1a	*Strong carbol fuchsin*	5 litres	Prepared from: Basic fuchsin powder 3g/l Phenol detached crystals 45g/l 95% methyl-alcohol 100ml/l Packed in 5 x 1 litre bottles
1b	*Acid alcohol 3% v/v*	5 litres	Prepared from: Concentrated hydrochloric acid 30ml/l 95% methyl-alcohol 970ml/l Packed in 5 x 1 litre bottles
1c	*Methylene Blue (3g/l)*	5 litres	Prepared from: Methylene blue chloride powder 3g/l Packed in 5 x 1 litre bottles
2	Industrialized methylated spirit	1 litre	For use in spirit lamp and for heating smears
3	Xylene	2.5 litres	For removing oil prior to slide storage for QC
4	Immersion oil	100ml	Non drying - Suitable for tropical countries. Medium viscosity, refractory index >1.5
5	Phenol disinfectant concentrate	5 litres	Lysol or equivalent phenolic disinfectant To be diluted to 5% working solution as per manufacturers instructions
6	Slides	1000	Microscope slides, washed glass, 76 x 26mm, 1.1 – 1.3 mm thick. Packed in 20 boxes of 50 slides or 10 boxes of 100 slides.
7	Filter paper	1 box/100 circles	Whatman No 1, 24 cm diameter
8	Lens cleaning tissue	2 pkts/25 tissues	
9	Instructions for use of stains	1	Manufacturer's instructions on stain content and use

2. CONSUMABLES KIT B

Contains materials sufficient to prepare and stain 1000 sputum smears

	Item ?	***Quantity per kit***	***Specification***
1	Basic fuchsin powder**	15 gram	*Colour index 42500-42510 max absorbance, +/- 665mm, biological stain. Pre-weighed in 5 packets of 3g.*
2	Phenol detached crystals**	225 gram	*Extra pure 99%. Pre-weighed in 5 air-tight containers, each containing 45g*
3	Methylene blue chloride powder**	15 gram	*Colour index 52015, max absorbance, +/- 665mm, certified stain. Pre-weighed in 5 packets of 3g.*
4.	Industrialised methylated spirit**	7.5 litres	*95% methyl-alcohol in 3 x 2.5 litre bottles Will be used for the following: 1) Preparation of 5 litres of strong carbol fuchsin 2) Preparation of 5 litres of acid-alcohol 3) For spirit lamp and heating smears*
5	Concentrated hydrochloric acid**	150ml	*For preparation of 5 litres of acid alcohol*
6	Xylene**	2.5 litres	*For removing oil prior to slide storage for QC*
7	Immersion oil**	100ml	*Non-drying, suitable for tropical countries. Medium viscosity, refractory index >1.5*
8	Phenol disinfectant concentrate **	5 litres	*Lysol or equivalent phenolic disinfectant To be diluted to 5% working solution as per manufacturers instructions*
9	Slides	1000	*Microscope slides, washed glass, 76 x 26mm, 1.1 – 1.3 mm thick. Packed in 20 boxes of 50 slides or 10 boxes of 100 slides.*
10	Filter paper	1 box/100 circles	*Whatman No 1, 24 cm diameter*
11	Lens cleaning tissue	2 pkts/25 tissues	
12	Instructions for preparation and use of stains	1	*Manufacturer's instructions*

*** With minimum expiry date of 2 years from time of delivery.*

3. SPUTUM COLLECTION CONTAINERS

For each Consumables Kit ordered, the GDF will automatically supply 1000 screw-capped, wide-mouth, disposable sputum collection containers, unless the applicant indicates that these are not required. The containers will be packed separately from the laboratory reagents in order to permit direct distribution to health facilities attended by TB suspects and patients for collection of sputum specimens. Sputum specimens can then be forwarded to the nearest microscopy centre.

Specification:
Screw cap, single use, combustible material, translucent with easily labelled wall panel. Wide mouth at least 45mm in diameter. Volume = 50 ml. Packed in cases of 1000

4. EQUIPMENT STARTER KIT A

Minor laboratory equipment for use with Consumables Kit A

	Item ?	***Quantity per kit***	***Specification***
1	WHO publications: Laboratory Services in Tuberculosis Control Part 1 Organization and Management and Part 2 Microscopy	1 of each book	Reference: WHO_TB_98.258
2	Laminated wall chart – Ziehl-Neelsen staining procedure	1	Either – to be prepared Or – use existing chart. e.g. from CDC Atlanta
3	Beaker	2	250ml, borosilicate, heavy duty, heavy banded rim, for general laboratory use
4	Filter funnel	2	Funnel, polypropylene, 150 or 160mm diameter
5	Oil dropper bottle	1	Polythene or other non-breakable material. Dropper to dispense 0.1 ml immersion oil
6	Wash bottles	2	Polythene, 500ml capacity
7	Stain dispensing bottles	3	250ml - dark, plastic, with spout and screw-on cap
8	Wire loop holder	2	Inoculating loop, Nichrome wire mounted in 18cm heat resistant handle.
9	Nichrome wire loops	75	Wire loops, volume 2.5 microlitres. Internal diameter = 2.5mm
10	Spirit lamp	2	Metal with screw cap, 60ml with complete cotton wick
11	Slide holding storage boxes	6	Plastic/100 slides per box
12	Marking pen	4	Black, waterproof magic marker
13	Slide drying rack	1	Wooden rack to hold 50 slides
14	Diamond slide marker	2	
15	Forceps	2	Slide holding forceps – 15cm

	Item ?	*Quantity per kit*	*Specification*
16	Staining rack	1	For use over laboratory sinks. Comprises two plated brass blocks each with two 7.5mm diameter holes to take glass rods to span sink and support microscope slide. With head screws for clamping glass rods, levelling screws and 2 glass rods + 1.5m.
17	Timer	1	Mechanical interval timer, for periods up to 60 min, the end of the period signalled by long bell ring. With indicator which can be turned to the required period and which winds and starts the clock mechanism

5. EQUIPMENT STARTER KIT B

Minor laboratory equipment for use with Consumables Kit B.

	Item ?	*Quantity per kit*	*Specification*
1	WHO publications: Laboratory Services in Tuberculosis Control Part 1 Organization and Management and Part 2 Microscopy	1 of each book	Reference: WHO_TB_98.258
2	Laminated wall chart – Ziehl-Neelsen staining procedure	1	Either – to be prepared Or – use existing chart. e.g. from CDC Atlanta
3	Beaker	2	250ml, borosilicate, heavy duty, heavy banded rim, for general laboratory use
4	Filter funnel	2	Funnel, polypropylene, 150 or 160mm diameter
5	Oil dropper bottle	1	Polythene or other non-breakable material. Dropper to dispense 0.1 ml immersion oil
6	Wash bottles	2	Polythene, 500ml capacity
7	Stain dispensing bottles	3	250ml - dark, plastic, with spout and screw-on cap
8	Wire loop holder	2	Inoculating loop, Nichrome wire mounted in 18cm heat resistant handle.
9	Nichrome wire loops	75	Wire loops, volume 2.5 microlitres. Internal diameter = 2.5mm
10	Spirit lamp	2	Metal with screw cap, 60ml with complete cotton wick
11	Slide holding storage boxes	6	Plastic/100 slides per box
12	Marking pen	4	Black, waterproof magic marker
13	Slide drying rack	1	Wooden rack to hold 50 slides
14	Diamond slide marker	2	
15	Forceps	2	Slide holding forceps – 15cm

	Item ?	*Quantity per kit*	*Specification*
16	Staining rack	1	For use over laboratory sinks. Comprises two plated brass blocks each with two 7.5mm diameter holes to take glass rods to span sink and support microscope slide. With head screws for clamping glass rods, levelling screws and 2 glass rods + 1.5m.
17	Timer	1	Mechanical interval timer, for periods up to 60 min, the end of the period signalled by long bell ring. With indicator which can be turned to the required period and which winds and starts the clock mechanism
18	Graduated measuring cylinders (100ml)	2	Cylinder, single scale, polypropylene - 100ml
19	Graduated measuring cylinders (500ml)	2	Cylinder, laboratory, single scale, polypropylene -500ml
20	Conical flask (2 litres)	2	Flask, narrow mouth, conical heat resistant glass (Pyrex), with two or more graduation marks
21	Conical flask (1 litres)	2	Flask, narrow mouth, conical heat resistant glass (Pyrex), with two or more graduation marks
22	Stirring rods	2	Solid glass rods
23	Stock bottles for stains	6	Brown glass reagent bottle 2.5l capacity with screw caps. ('Winchester' containers)

6. MICROSCOPE KIT with accessories

The microscope must have good quality optics, be robust and suitable for use in tropical countries. It is intended to be used with mains electricity. Because of continental differences in power supply, two specifications are required (a) for use with 210/250V and (b) for use with 110/125V. In addition, many countries have unreliable or intermittent power supplies. Voltage fluctuations, spikes, 'brown-outs' and sudden power cuts are common. Therefore a surge protector and a 12 volt battery (to provide a back-up power supply) will also be included. Two options will be provided for charging the battery – a mains charger and a solar panel.

	Item?	*Quantity*	*Minimum specification*
1	**Microscope**:	1	Binocular head, compensation free, rotatable 360° with built-in illumination; stand with coarse and fine focusing. Eyepieces inclined at 30°
	Eyepieces	2	Wide field, 10x. Each maintains parfocality at inter-pupillary distance from 55mm – 75mm and features dual eyepiece dioptre adjustments for maximum acuity.
	Objectives	4	4X, 10X, 40X (S) and 100X (S) (oil immersion). S= spring-loaded
	Nosepiece	1	Quadruple with RMS thread for objectives. Smooth and long lasting operation with 'click' stops at each objective position

	Item?	***Quantity***	***Minimum specification***
	Condenser	1	Abbe NA 1.25. Condenser to be mounted on a robust sub-stage with safety stop for critical positioning and centring. Condenser has adjustable iris diaphragm with swing-out filter holder
	Illumination	1	Built-in 10 watt Halogen Quartz lamp with variable brilliance control
	Power supply		2 specifications required. (a) 210/250V 50Hz and 12 V DC (b) 110/125V 6oHZ and 12 V DC
	Accessories:		
2	Surge protector	1	Automatic voltage switcher
3	12V battery unit	1	12 Volt, 6 amp, maintenance free lead acid battery unit which when fully charged provides approximately 3 hours of running time for the microscope. The unit can be charged from mains electricity and from a solar panel.
4	Mains charger	1	a) 210/250V b) 110/125V As per microscope specification
5	Solar panel	1	Amorphous silicon photovoltaic cells capable of providing 14.5 volts at a maximum charging current of 500ma. With 12 hours of direct sunlight, the panel will fully charge the battery unit. Without direct sunlight, a longer charge time will be required. The aluminium frame should be able to be angled to receive maximum sunlight.
6	Cable for solar panel	1	10 metre cable to connect battery unit to solar panel
7	Mirror	1	Mirror unit, interchangeable with the lamp unit. To be used as an alternative light source when mains or battery power is not available
8	Spare lamp	10	6V, 10 Watt, halogen quartz
9	Spare fuse	10	500mA
10	Dust cover	1	Washable
11	Storage box	1	Wooden, lockable
12	Self-indicating silica gel	100g	
13	Instruction manual	1	Manual must provide information on: 1) Setting up, routine use and maintenance of the microscope 2) Use of surge protector 3) How to switch between mains and battery power 4) How to charge the battery from both mains and solar panel 5) How to care for the battery 6) How to operate and care for the solar panel 7) How to interchange the mirror unit with the lamp unit

ANNEX 2

APPLICATION FORM FOR LABORATORY KITS
To be made available on the GDF website

Telephone Central/Exchange : (+41) 22 791 2111
Direct: (+41) 22 791 2385
E-mail: gdf@who.int
Fax: (+41) 22 791 4886

Our reference : Letter of Application/gov

To Whom It May Concern

Dear Sir/Madam,

09 June 2004

Application Form for grant of laboratory kits from the Global TB Drug Facility

In addition to supplying TB drugs, the Global TB Drug Facility (GDF) has also decided to provide TB diagnostics for sputum smear microscopy. I understand that you wish to avail of this new facility and be considered for a grant of laboratory kits from the GDF. In order to apply to the GDF for support, please complete the application form below. Additionally, please send, together with this application, the following documents:

Documents Required:

1. A covering letter explaining the need for a grant for laboratory kits.
2. Multi-year DOTS expansion plan to meet the global targets for TB control.
3. Most recent independent assessment/review of national TB control activities.
4. Most recent independent assessment/review of TB laboratory services
5. Technical and operational policies for the national TB programme.
6. National guidelines for AFB smear microscopy
7. Documentation on the AFB microscopy quality assurance and laboratory supervisory systems
8. Most recent annual report for case notifications and treatment outcome (WHO TB Data collection form) describing TB programme performance.
9. Evidence that national and international partners and/or donors support this application to the GDF (e.g. as co-signatories of the application).
10. Documentation describing flow of laboratory kits from time of receipt by the programme until use in the health facility including responsible persons

In order to ensure processing of this application by the GDF, and to confirm your agreement please sign and date *each page* of this application form and return it to Ms Lucy Moore, Global Partnership to Stop TB by fax (+ 41-22-7914486 or +41 22 791 4886) or email (moorel@who.int). If you have any queries or concerns relating to this letter, please do not hesitate to contact Dr Marcos Espinal, Executive Secretary, Global Partnership to Stop TB by fax (+41 22 791 4886), tel. (+41 22 791 2385) or email (espinalm@who.int).

Yours sincerely,

Dr M. Raviglione
Director, Stop TB

GLOBAL TB DRUG FACILITY
APPLICATION FORM FOR LABORATORY KITS

SECTION A. CONTACT DETAILS

Country:	
Contact person:	
Position:	
Address:	
Telephone:	
Fax:	
Email:	

SECTION B. COUNTRY INFORMATION

TB MICROSCOPY SERVICES		
1	Total number of smear microscopy units in your country	
2	Number of smear microscopy units at each level of the health system (Include both Government and NGO units providing TB microscopy):	
	LEVEL 1: Health Centre/PHC clinic	
	LEVEL 2: District hospital	
	LEVEL 3: Regional/Provincial hospital	
	LEVEL 4: Central laboratory	
	Other, please state ……..	
3	Number of smear microscopy units that received quarterly supervisory visits last year	
4	Number of smear microscopy units that actively participated in a national TB quality assurance scheme last year	
5	Estimated number of smear microscopy units that require basic laboratory equipment [Equipment Starter Kit A or B] (include new sites and existing units that are inadequately equipped)	
6	Estimated number of smear microscopy units that require a Microscope Kit (include new sites and existing units that are inadequately equipped)	
7	Total Number of TB suspects who had sputum examined in the last 12 months	
8	Total number of TB suspects who were diagnosed as 'smear positive' in the last 12 months	
9	Average annual national smear positivity rate	
10	Total number of smears examined in all microscopy centres last year	

Please indicate which type of support your require:

Emergency ☐ Regular ☐

Please indicate if you have already submitted an application to the Global Fund to Fight AIDS, Tuberculosis and Malaria, for TB support:

Yes ☐ No ☐

If yes, please indicate the date of the application and its outcome.

Date of the GFATM application: ……

Decision: Approved ☐

Please indicate below the value of the GFATM award (in US$), its duration and whether it includes monies for the procurement of laboratory consumables and equipment for TB microscopy

Decision: Not Approved ☐ Other (please explain below) ☐

Please list all other donors (multilateral, bilateral and NGOs) which are supplying TB laboratory consumables and equipment in your country or are providing monies for their procurement. Please indicate the type and value of their support (in US$) and its duration.

SECTION C. PRODUCTS FOR LABORATORIES

NOTES

- The ***Contents*** of each of the laboratory kits are listed in Annex 1
- ***Laboratory consumables kits (A or B)*** can be supplied by the GDF on annual basis for all TB microscopy units as required.
- ***Equipment Starter Kits (A or B)*** are expected to last for 3 years. ***Microscopes*** are expected to last a minimum of 5 years. After the initial supply, GDF can supply replacement Equipment Starter Kits every 3 years and microscopes every 5 years as required. Additional Equipment Starter Kits and microscopes can be ordered on an annual basis to equip new microscopy sites.

Products	Product required	Total Number of kits required for ONE YEAR
Laboratory Consumables Kit A Each kit contains 5 x 1 litre of ready-to-use Ziehl-Neelsen stains plus other consumables sufficient to process 1000 sputum specimens **NB: USE THE SPREAD SHEET ON THE FOLLWING PAGE TO CALCULATE THE NUMBER OF KITS REQUIRED**	YES ☐ NO ☐	
Laboratory Consumables Kit B Each kit contains dried reagents (pre-weighed) and chemicals for in-country preparation of 5 x 1 litre of Ziehl-Neelsen stain, plus other consumables sufficient to process 1000 sputum specimens	**NOT YET AVAILABLE**	
Sputum collection containers 1000 sputum containers will automatically be dispatched with every Consumables Kit (A or B) ordered, unless you indicate that you do **not** require sputum containers. DO YOU REQUIRE SPUTUM CONTAINERS?	YES ☐ NO ☐	
Equipment Starter Kit A Each kit contains minor items of equipment required to process and stain sputum specimens for AFB. This kit is intended to be used in conjunction with the Consumables Kit A.	YES ☐ NO ☐	
Equipment Starter Kit B In addition to the items contained in Equipment Starter Kit A, this kit contains items of glassware required to prepare the stains. This kit is intended to be used in conjunction with Consumables Kit B.	**NOT YET AVAILABLE**	
Microscope kit Each microscope kit contains one binocular microscope suitable for use both with mains electricity and a 12V battery (included), surge protector, spare bulbs and fuses	YES ☐ NO ☐	
Power supply. *Please indicate the type of power supply in your country:* 210/250V 60Hz ☐ 110/125V 50Hz ☐		

INSTRUCTIONS FOR CALCULATION OF CONSUMABLE KIT REQUIREMENTS

Calculation of the number of kits required is automatic. Please follow these instructions carefully:

1. Enter the total number of new smear positive patients diagnosed in the last 12 months in Column 1

2. Enter the average smear positivity rate in your country during the last 12 months in Column 2. Select one of the options: 10%, 15% or 20%. If your rate falls between these figures, select the nearest lower figure. For example if your smear positivity rate is 13.5%, then select 10%. If your rate is 17%, select 15%. IF THE RATE IS NOT KNOWN, SELECT 10%.

3. Enter the total number of consumables kits with expiry date not less than one year that you have in stock

1. **Total No of new smear positive patients diagnosed in the last 12 months**	**2.** **Average smear positivity rate during the last 12 months** ***Select one of the following options: 10%, 15%, 20%.***	**3.** **Factor**	**4.** **A = Total No of TB laboratory consumable kit A required for 1 year (Automatic calculation)**	**5.** **B = Required buffer stock for 1 year. Multiply A x 1 (Automatic calculation)**	**6.** **C = No of kits with expiry date not less than 1 year in central store**	**7.** **D = Total quantity of kits to be ordered = A + B - C (automatic calculation)**	**8.** **E = No of sputum containers to be supplied (Automatic calculation)**
a	10%	36	a x 36/1000	A		A + B - C	D x 1000
b	15%	27	b x 27/1000	A		A + B - C	D x 1000
c	20%	21	c x 21/1000	A		A + B - C	D x 1000

The calculations are based on the assumption that 5ml of each stain are needed for each sputum smear. Each kit will contain materials to prepare and stain 1000 slides. Kits will contain 5000ml of each stain and 1000 glass slides. 1000 sputum collection containers will automatically be supplied for each Consumable A kit ordered unless otherwise specified

Assumptions:

1) If **10% of examined TB suspects are smear positive,** each smear positive case will have a total of 9 slides examined (3 diagnostic, 2 at 2/12 follow-up, 2 x 5/12 follow-up and 2 at 7/12 follow-up). For each new smear positive case there will be 9 smear negative cases, each of whom will have 3 smears examined. Therefore for every smear positive case detected, a total of 36 smears will be examined.

2) If **15% of examined TB suspects are smear positive,** each smear positive case will have a total of 9 slides examined (3 diagnostic, 2 at 2/12 follow-up, 2 x 5/12 follow-up and 2 at 7/12 follow-up). For each new smear positive case there will be 6 smear negative cases, each of whom will have 3 smears examined. Therefore for every smear positive case detected, a total of 27 smears will be examined.

3) If **20% of examined TB suspects are smear positive**, each smear positive case will have a total of 9 slides examined (3 diagnostic, 2 at 2/12 follow-up, 2 x 5/12 follow-up and 2 at 7/12 follow-up). For each new smear positive case there will be 4 smear negative cases, each of whom will have 3 smears examined. Therefore for every smear positive case detected, a total of 21 smears will be examined.

Section D. CONSIGNEE DETAILS

1. Name and contact details of consignee (address, email, telephone, fax)	
2. Name and contact details of person/authority responsible for tracking TB laboratory order on internet (if different from above) [1] (*Please identify a person who can be contacted on a regular basis by the GDF procurement agent and provide a working telephone number and email address)*	
3. Name and contact details of person/authority responsible for laboratory materials registration issues	
4. Do you have any in-country partners who you would like to be kept informed of the GDF LABORATORY shipment? If yes, please provide name and email address.	
5. Preferred date(s) of delivery [2]	
6. Preferred port of delivery (air only)	
7. Special requirements concerning markings **8. Special requirements concerning documents e.g. airway bills, packing lists, invoices**	
9. Please confirm that no special pre-shipment inspections are required in addition to the pre-shipment inspection that will be carried out by the GDF agent. **10. Documentation needed to accompany consignment**	☐ No special requirements ☐ Special requirements (please describe): ☐ Airway bill ☐ Packing list ☐ Invoice ☐ Other (please describe):
11. Can shipment be made prior to registration of TB laboratory consumables (where applicable)? **12. Can shipment arrive outside of normal working hours?**	YES ☐ NO ☐ YES ☐ NO ☐
13. Advanced notice of shipment required (Please indicate contact person/authority to be notified, their complete mailing address, including e-mail and phone number, and required period of advanced notification)	YES ☐ NO ☐ Person/authority to be notified: Name: Mailing Address: E-mail: Tel. No.: Period of advance notification:

[1] Once the purchase order is issued for the TB diagnostic supplies, a copy will be posted on the GDF/IAPSO Web buy site. In order for you to be able to access and track the progress of this order on the Internet, UNDP-IAPSO will issue an Identification Number and Password, together with instructions on how to access the relevant information, to this contact person.

[2] The GDF aims to accommodate the delivery date(s) requested by applicantsbut cannot guarantee that delivery will occur, in every instance, on the date(s) requested. GDF expressly disclaims responsibility for any delays or defaults resulting from the acts or omissions of procurement or shipping agents, as well as for any delays or defaults caused by other conditions beyond its reasonable control, including, but not limited to Acts of God, Government restrictions (including the denial or cancellation of any export or other necessary license), wars, insurrections, fires, floods, or failure of any supplier or subcontractor substantially to meet its obligations to GDF.

14. Details of additional requirements	

SECTION E. SUPPORTING DOCUMENTS REQUIRED

Documents Required	**Provided**	**Indicate which documents may not be published by the GDF**
1. A covering letter explaining the need for a grant for laboratory kits.	☐	☐
2. Multi-year DOTS expansion plan to meet the global targets for TB control.	☐	☐
3. Most recent independent assessment/review of national TB control activities.	☐	☐
4. Most recent independent assessment/review of TB laboratory services	☐	☐
5. Technical and operational policies for the national TB programme.	☐	☐
6. National guidelines for AFB smear microscopy	☐	☐
7. Documentation on the AFB microscopy quality assurance and laboratory supervisory systems	☐	☐
8. Most recent annual report for case notifications and treatment outcome (WHO TB Data collection form) describing TB programme performance.	☐	☐
9. Evidence that national and international partners and/or donors support this application to the GDF (e.g. as co-signatories of the application).	☐	☐
10. Documentation describing storage and flow of laboratory kits from time of receipt by the programme until use in the health facility, including responsible persons	☐	☐

SECTION F. TERMS AND CONDITIONS OF GDF SUPPORT

1. All laboratory consumables and equipment supplied by the Global TB Drug Facility (GDF) will ONLY be used:

a. For diagnosis and monitoring of TB patients**
b. To provide AFB microscopy services free of charge to all patients
c. For laboratory diagnosis and patient monitoring following WHO guidelines
d. In programmes following national guidelines for DOTS implementation.
e. In accordance with a multi-year plan for DOTS expansion to reach global targets by 2005

** Where the workload permits, the microscope may be used within the laboratory to which it has been assigned, for other tests in addition to TB microscopy. This is on the understanding that it must always be made available as and when required for TB microscopy.

2. The applicant is responsible for the laboratory supplies and equipment beyond the agreed point of delivery. The applicant will make arrangements for the payment or waiver of any import duty or tax, storage fees or insurance levied on drugs and supplies from the GDF in a timely fashion so that the drugs and supplies are released by customs and supplied for programmatic needs as required. The applicant is responsible for the in-country storage, distribution and monitoring of laboratory supplies provided by the GDF.

3. Microscopes are expected to last a minimum of 5 years. They are supplied on the understanding that the applicant will be responsible to make the necessary local arrangements for routine cleaning, maintenance and annual professional servicing of all microscopes supplied by the GDF.

4. Where registration of laboratory materials is required, GDF laboratory reagents will be expeditiously registered and the applicant will facilitate this process, so that the items are released from registration and supplied for programmatic needs as required.

5. Regular assessments of the applicant's performance, including laboratory management, will be carried out by an independent technical agency, and the complete assessment report provided to the GDF. The applicant will also provide the following reports to the Stop TB secretariat:

1. A regular annual report on TB programme performance in accordance with WHO guidelines
2. Quarterly reports on case finding, smear conversion and treatment outcomes
3. A regular annual report on the laboratory quality assurance programme for TB microscopy
4. A regular annual report on management and supervision of TB microscopy units
5. A regular annual report on microscope maintenance and professional servicing
6. Date of arrival of GDF laboratory supplies at port
7. Time taken to register laboratory reagents (if applicable)
8. Date laboratory kits received in central medical store

6. Public sector/donor funding for TB control activities will not be reduced as a consequence of, or during the period that GDF grants are received.

7. Co-financing and technical co-operation are available from other partners/donors for non-laboratory aspects of the multi-year plan (including DOTS expansion).

Do you agree with the above mentioned terms and conditions of support? YES ☐ NO ☐

SECTION G. COUNTRY VISIT

In order to assist countries that have applied for GDF support for laboratory services, representatives of the Stop TB Partnership Secretariat, as well as Stop TB partners conduct country visits following review of applications. Please propose three suitable dates, in order of preference, for a country visit to monitor the management and operation of TB laboratory services.

1st choice

2nd choice

3rd choice

Please fill out the application form in French or English only, and send to the following address:

Ms Lucy Moore
Global TB Drug Facility
Stop TB Partnership Secretariat
c/o World Health Organization
20, avenue Appia
CH-1211 Geneva 27
Switzerland
Fax: +41-22-791 4486 or
+41-22-791 4886
Email: moorel@who.int

Annex 1 *(To GDF application form for Laboratory Kits)*

LABORATORY DIAGNOSTIC KITS

To promote DOTS expansion in low-income countries (GNP < US$3000) with high burden of TB, the Global TB Drug Facility will provide laboratory diagnostic kits to eligible countries free of charge. The kits will also be available for purchase at a reasonable cost to other partners who do not meet the eligibility criteria for free kits.

The following will be made available:

- Consumables Kit A (with ready-to-use Ziehl-Neelsen stains)
- Consumables Kit B (with chemicals and pre-weighed dry reagents for in-country preparation of Ziehl-Neelsen stains)
- Sputum collection containers
- Equipment Starter Kit A (to be used in conjunction with Consumables Kit A)
- Equipment Starter Kit B (to be used in conjunction with Consumables Kit B)
- Microscope kit

KIT CONTENTS:

1. Consumables Kit A
Contains materials sufficient to prepare and stain 1000 sputum smears

	Item ?	***Quantity***	***Specifications/Use***
1	Ready – to use Ziehl-Neelsen stains*:*		
1a	*Strong carbol fuchsin*	5 litres	Packed in 5 x 1 litre bottles
1b	*Acid alcohol 3% v/v*	5 litres	Packed in 5 x 1 litre bottles
1c	*Methylene Blue (3g/l)*	5 litres	Packed in 5 x 1 litre bottles
2	Industrialized methylated spirit	1 litre	For use in spirit lamp and for heating smears
3	Xylene	2.5 litres	For removing oil prior to slide storage for QC
4	Immersion oil	100ml	Suitable for tropical countries
5	Phenol disinfectant concentrate	5 litres	To be diluted to 5% working solution as per manufacturers instructions
6	Slides	1000	Microscope slides, washed glass, 76 x 26mm, 1.1 – 1.3 mm thick. Packed in 20 boxes of 50 slides or 10 boxes of 100 slides.
7	Filter paper	1 box/100 circles	Whatman No 1, 24 cm diameter
8	Lens cleaning tissue	2 pkts/25 tissues	

?Items required but not included: Cotton wool and disposable gloves. It is expected that these will be sourced locally by the NTP.

2. Consumables Kit B
Contains materials sufficient to prepare and stain 1000 sputum smears

	Item ?	*Quantity*	*Specification*
1	Basic fuchsin powder	15 gram	Pre-weighed in 5 packets of 3g. Each packet sufficient to make 1 litre of strong carbol fuchsin stain
2	Phenol detached crystals	225 gram	Pre-weighed in 5 air-tight containers. Each bottle contains 45g to make 1 litre strong carbol fuchsin stain
3	Methylene blue chloride powder	15 gram	Pre-weighed in 5 packets of 3g. Each packet sufficient to make 1 litre of methylene blue counterstain
4.	Industrialised methylated spirit	7.5 litres	95% methyl-alcohol in 3 x 2.5 litre bottles To be used for the following: 1) Preparation of 5 litres of strong carbol fuchsin (0.5 litres required) 2) Preparation of 5 litres of acid-alcohol (4.85 litres required) 3) For spirit lamp and heating smears
5	Concentrated hydrochloric acid	150ml	For preparation of 5 litres of acid alcohol (30ml HCl per litre)
6	Xylene	2.5 litres	For removing oil prior to slide storage for QC
7	Immersion oil	100ml	Suitable for tropical countries
8	Phenol disinfectant concentrate	5 litres	To be diluted to 5% working solution as per manufacturers instructions
9	Slides	1000	Microscope slides, washed glass, 76 x 26mm, 1.1 – 1.3 mm thick. Packed in 20 boxes of 50 slides or 10 boxes of 100 slides.
10	Filter paper	1 box/100 circles	Whatman No 1, 24 cm diameter
11	Lens cleaning tissue	2 pkts/25 tissues	

*?*Items required but not included: Cotton wool and disposable gloves. It is expected that these will be sourced locally by the NTP.

3. Sputum collection containers

For each Consumables Kit ordered, the GDF will automatically supply 1000 screw-capped, wide-mouth, disposable sputum collection containers, unless the applicant indicates that these are not required. The containers will be packed separately from the laboratory reagents in order to permit direct distribution to health facilities attended by TB suspects and patients for collection of sputum specimens. Sputum specimens can then be forwarded to the nearest microscopy centre.

4. Equipment Starter Kit A
Minor laboratory equipment for use with Consumables Kit A

	Item ?	*Quantity*	*Specification*
1	WHO publications: Laboratory Services in Tuberculosis Control Part 1 Organization and Management and Part 2 Microscopy	1	Reference: WHO_TB_98.258
2	Laminated wall chart – Ziehl-Neelsen staining procedure	1	
3	Beaker	2	250ml, borosilicate, heavy duty, heavy banded rim, for general laboratory use
4	Funnel	2	Funnel, polypropylene, 150 or 160mm diameter
5	Oil dropper bottle	1	Dropper to dispense 0.1 ml oil
6	Wash bottles	2	Polythene, 500ml capacity
7	Stain dispensing bottles	3	Dark, plastic, with spout and screw-on cap
8	Wire loop holder	2	Inoculating loop, Nichrome wire mounted in 18cm heat resistant handle.
9	Nichrome wire loops	75	Wire loops, volume 2.5 microlitres. Internal diameter = 2.5mm
10	Spirit lamp	2	Metal with screw cap, 60ml with complete cotton wick
11	Slide holding storage boxes	6	Plastic/100 slides per box
12	Waterproof marking pen	4	Black
13	Slide drying rack	1	Wooden rack to hold 50 slides
14	Diamond slide marker	2	
15	Forceps	2	Slide holding forceps – 15cm
16	Staining rack	1	For use over laboratory sinks. Comprises two plated brass blocks each with two 7.5mm diameter holes to take glass rods to span sink and support microscope slide. With head screws for clamping glass rods, levelling screws and 2 glass rods + 1.5m.
17	Timer	1	Mechanical interval timer, for periods up to 60 min, the end of the period signalled by long bell ring

5. Equipment Starter Kit B
Minor laboratory equipment for use with Consumables Kit B.

	Item ?	*Quantity*	*Specification*
1	WHO publications: Laboratory Services in Tuberculosis Control. Part 1 Organization and Management and Part 2 Microscopy	1	Reference: WHO_TB_98.258
2	Laminated wall chart – Ziehl-Neelsen staining procedure	1	
3	Beaker	2	250ml, borosilicate, heavy duty, heavy banded rim, for general laboratory use
4	Funnel	2	Funnel, polypropylene, 150 or 160mm diameter
5	Oil dropper bottle	1	Dropper to dispense 0.1 ml oil
6	Bottles	2	Polythene, 500ml capacity
7	Stain dispensing bottles	3	Dark, plastic, with spout and screw-on cap
8	Wire loop holder	2	Inoculating loop, Nichrome wire mounted in 18cm heat resistant handle.
9	Nichrome wire loops	75	Wire loops, volume 2.5 microlitres. Internal diameter = 2.5mm
10	Spirit lamp	2	Metal with screw cap, 60ml with complete cotton wick
11	Slide holding storage boxes	6	Plastic/100 slides per box
12	Waterproof marking pen	2	Black
13	Slide drying rack	1	Wooden rack to hold 50 slides
14	Diamond slide marker	2	
15	Forceps	2	Slide holding forceps 15cm
16	Staining rack	1	For use over laboratory sinks. Comprises two plated brass blocks each with two 7.5mm diameter holes to take glass rods to span sink and support microscope slide. With head screws for clamping glass rods, levelling screws and 2 glass rods + 1.5m.
17	Timer	1	Mechanical interval timer, for periods up to 60 min, the end of the period signalled by long bell ring
18	Graduated measuring cylinders (100ml)	2	Cylinder, single scale, polypropylene - 100ml

	Item ?	*Quantity*	*Specification*
19	Graduated measuring cylinders (500ml)	2	Cylinder, laboratory, single scale, polypropylene -500ml
20	Conical flask (2 litres)	2	Flask, narrow mouth, conical heat resistant glass (pyrex), with two or more graduation marks
21	Conical flask (1 litres)	2	Flask, narrow mouth, conical heat resistant glass (pyrex), with two or more graduation marks
22	Stirring rods	2	Solid glass rods
23	Stock bottles for stains	6	Brown glass reagent bottle 2.5l capacity ('Winchester' containers)

6. Microscope Kit

The microscope is suitable for tropical countries and is intended to be used with mains electricity (either 210/250V or 110/125V). Because many countries have unreliable or intermittent power supplies, a surge protector and a 12 volt battery (to provide a back-up power supply) will also be included. The battery can be charged either from the mains or from a solar panel.

	Item ?	*Quantity*	*Specification*
1	**Binocular microscope**:	1	Power supply, either 210/250V or 110/125V
	Eyepieces	2	Widefield, 10x.
	Objectives	4	4X, 10X, 40X and 100X (oil immersion)
	Nosepiece	1	Quadruple
	Condenser	1	Abbe NA 1.25
	Illumination	1	Built-in 10 Watt Halogen Quartz lamp
	Accessories:		
2	Surge protector	1	Automatic voltage switcher
3	12V battery unit	1	12 Volt, 6 amp, maintenance free battery unit.
4	Mains charger	1	Either 210/250V or 110/125V
5	Solar panel	1	Capable of providing 14.5 volts to charge the 12V battery
6	Cable for solar panel	1	10 metre cable to connect battery to solar panel
7	Mirror unit		Interchangeable with lamp unit
8	Spare lamp	10	6V, 10 Watt, halogen quartz
9	Spare fuse	10	500mA
10	Dust cover	1	Washable
11	Storage box	1	Wooden, lockable
12	Self-indicating silica gel	100g	
13	Instruction manual	1	

ANNEX 3

ALGORITHMS TO QUANTIFY ORDER REQUIREMENTS FOR CONSUMABLES KITS

Based on the number of smear positive cases detected and smear positivity rate

ALGORITHMS FOR CALCULATION OF CONSUMABLE KIT REQUIREMENTS

Enter the total No of new smear positive patients diagnosed in the last 12 months	**Average smear positivity rate in your country during the last 12 months - s*elect one of the following options: 10%, 15%, 20%.* IF THE RATE IS NOT KNOWN, SELECT 10%**	**Factor**	**A = Total No of TB laboratory consumable kit A required for 1 year**	**B = Required buffer stock. Multiply A x 1**	**C = No of kits with expiry date not less than 1 year in central store**	**D = Total quantity of kits to be ordered = A + B - C)**	**E = No of sputum containers to be supplied**
a	10%	36	a x 36/1000	A		A + B - C	D x 1000
b	15%	27	b x 27/1000	A		A + B - C	D x 1000
c	20%	21	c x 21/1000	A		A + B - C	D x 1000
Example:							
2000	10%	36	2000 x 36/1000 = 72 kits	72	0	**144**	144,000
2000	15%	27	2000 X 27/1000 = 54 kits	54	0	**108**	108,000
2000	20%	21	2000 x 21/1000 = 42 kits	42	0	**84**	84,000

The calculations are based on the assumption that 5ml of each stain are needed for each sputum smear. Each kit will contain materials to prepare and stain 1000 slides. Kits will contain 5000ml of each stain and 1000 glass slides. 1000 sputum collection containers will automatically be supplied for each Consumable A kit ordered unless otherwise specified

Assumptions:

1) If **10% of examined TB suspects are smear positive,** each smear positive case will have a total of 9 slides examined (3 diagnostic, 2 at 2/12 follow-up, 2 x 5/12 follow-up and 2 at 7/12 follow-up). For each new smear positive case there will be 9 smear negative cases, each of whom will have 3 smears examined. Therefore for every smear positive case detected, a total of 36 smears will be examined.

2) If **15% of examined TB suspects are smear positive,** each smear positive case will have a total of 9 slides examined (3 diagnostic, 2 at 2/12 follow-up, 2 x 5/12 follow-up and 2 at 7/12 follow-up). For each new smear positive case there will be 6 smear negative cases, each of whom will have 3 smears examined. Therefore for every smear positive case detected, a total of 27 smears will be examined.

3) If **20% of examined TB suspects are smear positive**, each smear positive case will have a total of 9 slides examined (3 diagnostic, 2 at 2/12 follow-up, 2 x 5/12 follow-up and 2 at 7/12 follow-up). For each new smear positive case there will be 4 smear negative cases, each of whom will have 3 smears examined. Therefore for every smear positive case detected, a total of 21 smears will be examined.

ANNEX 4

COMPOSITION OF INDIVIDUAL STAINS AND METHODS FOR THEIR PREPARATION

Consistent with WHO guidelines

Composition and preparation of stains for the Ziehl-Neelsen staining method

1). Strong carbol fuchsin stain

Fuchsin solution

Basic Fuchsin powder, Colour index 42500-42510, max absorbance, +/- 665mm, biological stain	3g
95% methyl alcohol (technical grade)	100ml

Dissolve basic fuchsin in the 95% methyl alcohol – SOLUTION 1

Phenol solution:

Phenol detached crystals, extra pure 99%	45g
Distilled water	900ml

Dissolve phenol crystals in distilled water – gentle heat may be required – SOLUTION 2

Strong carbol fuchsin

Combine 100ml of SOLUTION 1 with 900ml of SOLUTION 2. Filter and store in an amber bottle. Label with name of reagent, batch number, dates of preparation and expiry. Filter again immediately before use.

2). Acid alcohol (3% v/v) decolourising agent

Concentrated Hydrochloric Acid (technical grade)	30ml
95% methyl alcohol (technical grade)	970ml

Carefully add the concentrated hydrochloric acid to the 95% methyl alcohol. The mixture will heat up – so always add acid **slowly** to alcohol, not vice versa.
Store in an amber bottle. Label with name of reagent, batch number, dates of preparation and expiry

3). Methylene Blue (3%) counterstain

Methylene blue chloride powder, colour Index 52015, max absorbance, +/- 665mm, certified stain	3g
Distilled water	up to 1000 ml

Dissolve methylene blue chloride powder in distilled water. Filter and store in an amber bottle. Label with name of reagent, batch number, dates of preparation and expiry

ANNEX 5

STANDARD LABORATORY PROCEDURES FOR TB MICROSCOPY, USING THE ZIEHL-NEELSEN STAINING METHOD

Method consistent with WHO guidelines

Laboratory Procedures for TB Microscopy

Method
Ziehl Neelsen (ZN) stain

Purpose
To identify acid-fast bacilli in sputum smears.

Principle
Mycobacteria have the ability to resist decolorisation in the presence of a weak mineral acid after staining with an arylmethane dye. In the ZN staining method, carbol fuchsin, combined with phenol, binds to the mycolic acid in the mycobacterial cell wall. After staining, acid alcohol removes the red dye from the background cells, tissue fibres and all other organisms in the smear except the mycobacteria, which retain the dye. Methylene blue is added as a counter stain. The acid fast bacilli will stain red and the non-acid fast bacteria including the background, will stain blue.

Specimens
Three sputum specimens are collected from each TB suspect. The first sample is taken on the spot (usually in the consulting room), the second sample must be an early morning sample and the third is taken on the spot when the client returns to the consulting room with the second sample.

The sputum must be collected into a dry, clean, transparent, wide-mouthed leak proof container. THE SIDES OF THE SPECIMEN CONTAINER SHOULD BE LABELLED correctly with the patient's full name, age, address/clinic/ward and date and time of collection.

Test procedures

a) Smear preparation
1. Match up the request forms and the sputum containers.
2. Describe the appearance of each sputum specimen (e.g. purulent, blood stained etc.) and write the findings on the request form.
3. Enter the particulars of each form in the TB register and give specimens and form a consecutive TB register number.
4. For each specimen take a new slide and label it with patient's TB register number and specimen number (1,2 or 3) using the diamond pencil.
5. Flame the loop red hot and allow it to cool
6. With the inoculating loop, pick out mucopurulent or blood stained particles from the container and prepare a smear on the centre of a slide to cover an area of 2 x 1 cm
7. To determine the correct thickness of the film, place the slide on a piece of newspaper. Small prints should just be visible through the smear.
8. Flame the loop red hot and cool it before making the next smear.
9. Leave prepared smears on the bench to air dry for 15 - 30 minutes. Do **NOT** use the flame for drying the smears.
10. Fix the dry smears in the flame. Hold each slide at the labelled end with the slide forceps. With the smear on top, pass it 3 times through the flame of the spirit lamp. This should take 3 - 5 seconds.
11. Put the smears on staining rack.

b) ZN Staining Procedure
1. Cover the smears with filtered strong carbol fuchsin stain.
2. With a cotton swab soaked in spirit, heat the stain until vapour just begins to rise (i.e. about 60°C). **Do not overheat.**

3. Allow the heated stain to remain on the slide for 5 minutes.
4. Wash off the stain with clean water.
5. Cover the smear with 3% v/v acid alcohol for 5 minutes or until the smear is sufficiently decolourised, i.e. pale pink.
6. Wash well with clean water.
7. Cover the smear with methylene blue stain for 1-2 minutes.
8. Wash well with clean water.
9. Wipe the back of the slide and place it in a draining rack for the smear to air dry. **Do not blot dry**.

c) Microscopy

1. Examine smears under the microscope using x100 (oil immersion) objective.
2. Examine at least 100 fields.

Results

Presence of pinkish red rods = presence of acid fast bacilli
Absence of pinkish red rods = absence of acid fast bacilli

Interpretation and reporting

Score the findings using the standard WHO/IUATLD grading chart, as indicated below:

AFB counts	***Recording and reporting***
No AFB seen in at least 100 fields.	0
1 - 9 AFB in 100 fields	Report the exact number
10 - 99 AFB in 100 fields.	+
1 - 10 AFB per field in at least 50 fields.	++
Greater than 10 AFB per field in at least 20 fields	+++

Record the results on the laboratory form and in the laboratory TB register.

Quality Control

- Stain must not boil or dry on the slide.
- Use new, grease-free, glass slides without scratches.
- Label glass slides with diamond pencil to avoid the label coming off.
- Slides used for sputum smears must never be re-used.
- After examining every positive slide, the oil immersion objective must be cleaned to avoid transferring bacilli to the next slide.
- Newly prepared stains or new batches of ready-to-use stains must be controlled with known positive and negative slides.
- All the slides examined should be kept for external quality control in accordance with local instructions from the NTP.

Safety precautions

- Ideally all sputum specimens should be processed in a Class 1 safety cabinet. Where these cabinets are not available, conduct smear preparation in a well-ventilated area and avoid aerosol formation by using proper techniques for smear preparation.
- Hydrochloric acid is corrosive and must be handled with care. Store away from direct light.
- Carbol fuchsin is carcinogenic - wear gloves.
- Used sputum samples should be disinfected with 5% phenol disinfectant or autoclaved prior to incineration. If there is no incinerator, they may be disposed of in a closed pit.